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Osmotic stress induces biofilm production by *Staphylococcus epidermidis* isolates from neonates

Rosana B.R. Ferreira [a], Marcelle C.S. Ferreira [a], Thaís Glatthardt [a], Myllena Pereira Silvério [a], Raiane C. Chamon [b], Vivian C. Salgueiro [a], Lorrayne Cardoso Guimarães [a], Eduardo Souza Alves [a], Kátia R.N. dos Santos [a,*]

[a] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil
[b] Faculdade de Medicina, Universidade Federal Fluminense, Rio de Janeiro, Brazil

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ABSTRACT

Staphylococcus epidermidis is one of the leading causes of bloodstream infections, particularly in premature neonates, and biofilm formation is a major virulence factor. We characterized biofilm formation by 50 *S. epidermidis* neonatal isolates under osmotic stress and evaluated the expression of biofilm-associated genes. Phenotypical analyses of biofilm production were performed in culture medium with or without addition of NaCl or glucose. In control medium (no additions), most isolates (84%) were nonproducers or weak biofilm producers. Growth in NaCl-containing medium increased the number of moderate/strong producers, and this increase was even greater in medium containing glucose. Most of the protein-enriched biofilms (60%) could be observed only during growth in glucose, whereas 50% of the polysaccharide-enriched biofilms were observed during growth in NaCl. Studies that evaluate the conditions used to characterize biofilm production are important to help us understand the dynamics of this important virulence factor in *S. epidermidis* and their impact on neonatal infections.

1. Introduction

Despite being a member of the human microbiota, *Staphylococcus epidermidis* is described as a major nosocomial pathogen isolated from bloodstream infections (Iorio et al., 2011; Otto, 2009). This microorganism is usually isolated from infections associated with catheters and other medical devices, and neonates and immunocompromised individuals are the main patient groups involved (Kleinschmidt et al., 2015; Otto, 2009). The immature immune system of neonates, particularly of those born prematurely and admitted to neonatal intensive care units (NICUs), can increase the risk of infections by *S. epidermidis* (Hooven and Polin, 2014; Salgueiro et al., 2014; Wójkowska-Mach et al., 2014).

S. epidermidis pathogenesis is mostly associated with biofilm production, where bacterial cells aggregate in layers embedded in an extracellular matrix. Initial bacterial adhesion occurs in both artificial polymeric and human extracellular matrix surfaces. Adhesion to abiotic surfaces is mediated by *S. epidermidis* proteins Aap, Atl, and/or Bhp. During infection, bacterial cells interact with fibrinogen, fibronectin, collagen, and/or vitronectin, which are produced by the host and deposited on the surface of medical devices (Otto, 2013). Biofilm maturation starts with intercellular aggregation, in which an extracellular matrix, composed by polysaccharide, proteins, and/or extracellular DNA, is formed between cells (Arciola et al., 2015; Otto, 2013). Polysaccharide-enriched matrices are described as the most frequent extracellular matrix type in *S. epidermidis* clinical isolates, and they are composed mainly of polysaccharide intercellular adhesin (PIA) (Vuong et al., 2004), which is encoded within the *ica* (*intercellular adhesion*) operon. However, biofilm-producing *S. epidermidis* isolates without *ica* genes have also been described, and their biofilms are composed mainly of proteins, including Aap, Bhp, and Embp (Juárez-Verdayes et al., 2013; Rohde et al., 2005; Schaeffer et al., 2015; Speziale et al., 2014). Extracellular DNA also appears to have a significant role in these biofilms (Payne and Boles, 2016).

Research on *S. epidermidis* biofilm production under different conditions is important to better understand the role of environmental conditions during biofilm formation. Previous studies have shown that different concentrations of glucose or NaCl can induce stronger biofilm production by *S. epidermidis* isolates from healthy skin and meningitis (Barbieri et al., 2015; Cerca et al., 2011). However, most studies were performed using only a few isolates, and therefore, the conclusions that can be drawn from them are limited. Additionally, further studies

* Corresponding author. Tel.: +55-21-2560-8344, fax: +55-21-2560-8028.
E-mail addresses: rferreira@micro.ufrj.br (R.B.R. Ferreira), marcellec.ferreira@yahoo.com.br (M.C.S. Ferreira), glatthardt.thais@gmail.com (T. Glatthardt), myllenapsss@gmail.com (M.P. Silvério), raianee@gmail.com (R.C. Chamon), vivi.salgado@gmail.com (V.C. Salgueiro), lorraynecardog@yahoo.com.br (L.C. Guimarães), eduardoalvesufrj@gmail.com (E.S. Alves), santoskrn@micro.ufrj.br (K.R.N. dos Santos).

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are required to determine if these observations also apply to other types of isolates, such as those obtained from neonatal bloodstream infections. Here, we describe an analysis of the impact of osmotic stress on biofilm formation in a collection of *S. epidermidis* strains isolated from bloodstream infections and nasal colonization from neonates hospitalized in NICUs in Rio de Janeiro. We also evaluated if there is a correlation between the type of osmotic stress that induced biofilm formation and the type of biofilm matrix detected, as well as the impact of such stress on *ica* and *aap* gene expression.

2. Materials and methods

2.1. Bacterial strains

S. epidermidis isolates were obtained between May 2007 and March 2012 from neonates hospitalized in NICUs in hospitals in Rio de Janeiro, Brazil. All 50 isolates studied, including 31 isolates from bloodstream infections and 19 isolates from nasal colonization, were previously characterized by our group regarding their genetic background, presence of virulence and resistance genes, as well as biofilm production in TSB medium (Table 1; Salgueiro et al., 2017).

2.2. Biofilm quantification in microtiter plates

Measurements of biofilm production by all isolates were performed according to Stepanovic et al. (2007), with modifications. Biofilm production was analyzed after growth on TSB medium, TSB supplemented with 4% NaCl (TSB-NaCl), and TSB supplemented with 1% glucose (TSB-Glu) to evaluate the effect of osmotic stress on biofilm production. Briefly, colonies grown on 5% blood agar medium were suspended in sterile distilled water to reach the 0.5 McFarland scale. Then, 20 μL of the bacterial suspension was transferred to wells of a flat-bottom polystyrene microplate containing 180 μL of the specific culture medium, and plates were incubated at 37 °C for 24 h without shaking. After bacterial growth, wells were washed twice with sterile PBS buffer (pH 7.2), dried for 1 h at 60 °C, and stained with 200 μL of a 0.1% safranin solution (*w*/*v*, in water) for 15 min. Then, wells were washed twice again, and 200 μL of a 95% ethanol solution was added. Absorbance (OD_{492nm}) was read after 30 min of incubation at room temperature. Data analyses were performed according to Stepanovic et al. (2007), and the isolates were classified as nonproducers, weak, moderate, or strong producers. All biofilm experiments were performed in triplicate at 3 independent times.

2.3. Determination of biofilm chemical composition

S. epidermidis isolates previously characterized as biofilm producers in at least 1 of the tested conditions were analyzed to determine the chemical composition of their biofilms (Frank and Patel, 2007). *S. epidermidis* ATCC 35984 was used as the control for polysaccharidic biofilms, and *S. lugdunensis* 546s (Pereira et al., 2012) was used as the control for proteic biofilms. Bacterial growth was obtained as described for the biofilm production analyses. Biofilms were then treated with the following reagents: sodium metaperiodate ($NaIO_4$, 40 mM) or proteinase K (100 μg/mL), both in Tris–HCl 10 mM (pH 7.5). Proteinase K inactivated by boiling for 40 min and PBS buffer were used as negative controls. Plates were incubated at 37 °C for 2 h, washed twice with PBS buffer, and dried for 40 min at room temperature. Then, 100 μL of 0.1% safranin was added, and plates were incubated at room temperature for 15 min. After washing with PBS buffer, the plates were dried at room temperature for 40 min, 200 μL of 95% ethanol was added, and the absorbance was read at 492 nm. The treatment causing the greatest reduction in the final absorbance compared to the PBS control determined the chemical nature of the biofilm. In cases where proteinase K treatment caused the greatest biofilm reduction, the biofilm was considered proteic. Alternatively, whenever $NaIO_4$ caused the greatest reduction in biofilm formation, the biofilm was considered polysaccharidic.

Table 1
Characteristics of the *Staphylococcus epidermidis* neonatal isolates used in this study.

Isolation site (*n*)	Biofilm-associated genes *n* (%)			
	ica+/*aap*−	*ica*+/*aap*+	*ica*−/*aap*+	*ica*−/*aap*-
Blood (31)	7 (22.6)	7 (22.6)	9 (29)	8 (25.8)
Nares (19)	0 (0)	10 (52.6)	6 (31.6)	3 (15.8)

2.4. Gene expression analysis by real-time qPCR

Expression of *ica* and *aap* genes in sessile cells was tested using a ica^+ and aap^+ *S. epidermidis* isolate (number 810) that produced biofilms only under osmotic pressure. The following primers were used to analyze *ica*A and *app* gene expression: icaAFw: TCTCTTGCAGGAGCAATCAA; icaRev: AGGCACTAACATCCAGCA; aapFw: AGAAACAAGCTGGTCAAG; and aapRev: CTGCGTAGTTAAGAAAATC (Juárez-Verdayes et al., 2013).The *gmk* gene, which encodes the guanylate kinase enzyme, was used as the endogenous control for the reactions, and primers used were: gmkFw: TCAGGTGTTGGAAAGGGAAC and gmkRev: CGCCTCAAATTCTTCCTTTG (Linnes et al., 2013).

Bacterial growth was performed as described for biofilm production analyses. After incubation, each well was washed twice with PBS buffer followed by the addition of 1 mL of RNAprotect Bacteria Reagent (Qiagen, Hilden, Germany). Biofilms were scrapped using a cell scraper and transferred to a microtube. Bacterial suspensions were then mixed for 5 s by vortexing and incubated at room temperature for 5 min. The samples were centrifuged at 12,000 ×*g* for 10 min at 20 °C and 200 μL of lysis solution (containing 20 mg/mL of lysozyme in TE buffer and 10 μL of lysostaphin at 1 mg/mL) and 50 μL of proteinase K (20 mg/mL) were added to the pellet. Then, samples were mixed by vortexing for 10 s and incubated for 1 h at 37 °C, mixing every 10 min. For mechanical lysis of the bacterial cells, 700 μL of RLT buffer (RNeasy Mini Kit, Qiagen) containing 1% of β-mercaptoethanol (Sigma, Saint Louis, USA) was added to the suspensions, which were mixed and transferred to a microtube containing glass beads (106 microns and finer, acid-washed, Sigma). Tubes were mixed for 5 s and submitted to agitation for 3 times in a Mini-BeadBeater-16 (BioSpec Products, Bartlesville, USA). Later, samples were centrifuged in maximum speed for 10 min, and 500 μL of 100% ethanol was added to the collected supernatant. RNA purification was then performed using the RNeasy Mini Kit (Qiagen), as proposed by the manufacturer. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's manual. qPCRs were performed using Power SYBR Green PCR Master Mix (Applied Biosystems) and an ABIPrism 7500 (Applied Biosystems) thermocycler. Sample CTs (threshold cycles) were compared with the CTs of the reference sample (ATCC 35984), and the expression of target genes was calculated and normalized by both the endogenous control and the reference sample expression levels.

3. Results

3.1. Biofilm production under osmotic stress conditions

The ability to produce biofilms of a well-characterized collection of 50 *S. epidermidis* isolates from neonates hospitalized in NICUs was previously described by our group in TSB medium (Salgueiro et al., 2017). Most of the isolates (34, 68%) were classified as nonproducers under these conditions, whereas 8 (16%) isolates were classified as weak producers, 1 (2%) was classified as moderate producer, and 7 (14%) as strong biofilm producers. Here, we sought to analyze the impact of

high osmolarity on in vitro biofilm formation by these isolates (Fig. 1A). When NaCl (4%) was added to the growth medium (TSB), a decrease in the number of nonproducers was observed (24, 48%), whereas the number of isolates classified as weak (12, 24%), moderate (2, 4%), and strong (12, 24%) producers increased. Addition of glucose to the culture medium had an even stronger effect on biofilm production by these isolates; under this condition, only 13 (26%) isolates were classified as nonproducers, whereas 14 (28%) isolates were weak, 10 (20%) were moderate, and 13 (26%) were strong biofilm producers.

In contrast to biofilm production in TSB, where only 16 (32%) isolates were classified as biofilm producers, 41 (82%) isolates produced biofilm in at least 1 of the test conditions (NaCl or glucose supplementation), indicating that osmotic stress, regardless of its nature, has a significant impact on biofilm production by *S. epidermidis*. When comparing isolates from bloodstream infections and nasal colonization, we observed that 23 out of the 31 isolates (74.2%) from infections produced biofilms in at least 1 of the conditions tested. On the other hand, among the 19 isolates from colonization (nares), 18 (94.7%) produced biofilms under at least 1 of the growth conditions, indicating that isolates from nasal colonization of neonates had a slightly increased probability of being biofilm producers. Even though isolates from nasal colonization produced biofilms more often than isolates from blood infections, osmotic stress enhanced biofilm formation on a similar manner in both groups. Among the 31 isolates from infection, 20 (64.5%) produced stronger biofilms after osmotic stress; of these, 80% were induced by the addition of glucose, whereas 55% were induced by the addition of NaCl. Among the 19 colonization isolates, supplementation with either NaCl or glucose increased biofilm production of 13 (68.4%) isolates.

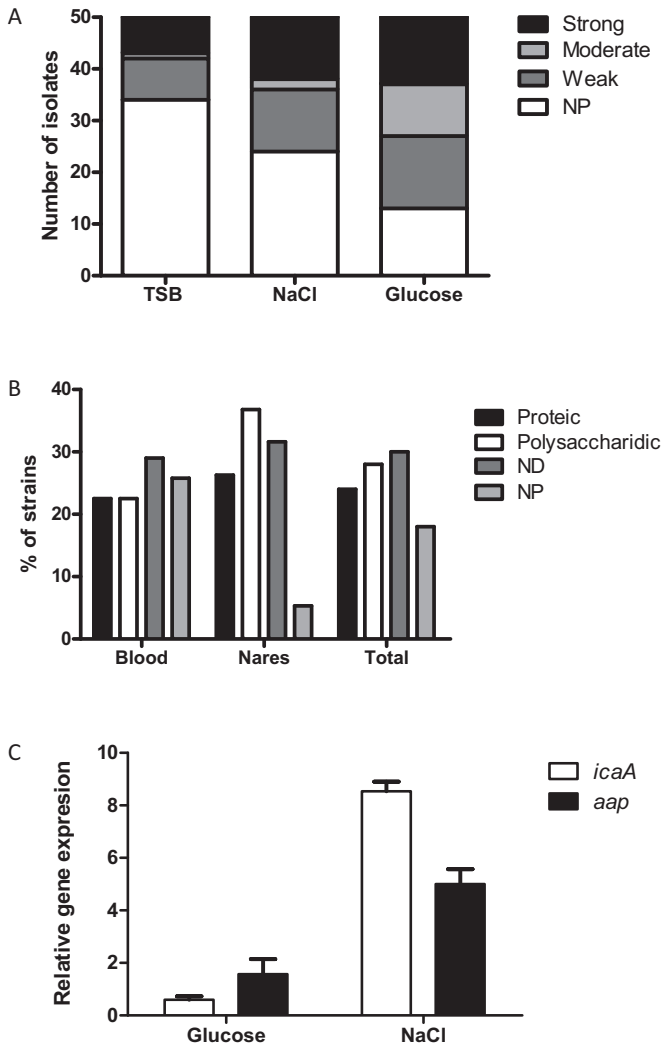

Fig. 1. Biofilm and gene expression analysis of *Staphylococcus epidermidis* isolates grown under osmotic stress. (A) Biofilm production of *S. epidermidis* isolates in different conditions. (B) Matrix composition of isolates according to isolation site; (C) relative *ica* and *aap* gene expression on biofilms from an *S. epidermidis* isolate after growth in media with 1% glucose or 4% NaCl. TSB = tryptic soy broth medium; 4% NaCl = TSB with addition of NaCl at 4%; 1% glucose = TSB with addition of glucose at 1%; NP = nonproducer; ND = not defined.

3.2. Biofilm matrix composition of S. epidermidis isolates

The main composition of biofilm matrices produced under the condition that stimulated the strongest phenotype for each isolate was analyzed. Among all *S. epidermidis* isolates that produced biofilm in at least 1 of the conditions ($n = 41$), biofilm matrix composition analyses revealed that 12 (29.3%) had a protein-enriched biofilm and 14 (34.2%) produced polysaccharide-based biofilms. The composition could not be determined for the remaining 15 (36.5%) isolates. When we analyzed the matrix composition of isolates from infections ($n = 23$), we found that 7 (30.4%) of them were proteinaceous, whereas the other 7 (30.4%) had polysaccharide-enriched biofilms. We were not able to determine the matrix composition for the remaining 9 (39.2%) isolates. Among the isolates from nares ($n = 18$), the biofilm matrix was classified as protein-based in 5 (27.8%) isolates, polysaccharide-based in 7 (38.9%), and undefined in the remaining 6 (33.3%) isolates (Fig. 1B). On all occasions, the production of protein-enriched biofilms was stronger when *S. epidermidis* was grown in TSB-Glu, whereas polysaccharide-enriched biofilms were more pronounced when isolates were grown in TSB-NaCl.

3.3. Relationship between ica and aap genes, biofilm production, and matrix composition

The *S. epidermidis* isolates used in this study were previously characterized regarding the presence of *ica* and *aap* virulence genes (Salgueiro et al., 2017). Therefore, we sought to compare these data with the data on biofilm matrix composition described herein in order to determine if there is an association between these two microbial characteristics. Among isolates that produced a protein-enriched biofilm either in TSB or TSB-Glu ($n = 10$), the majority (90%) was *aap* positive (6 aap^+ and 3 aap^+ica^+). The remaining isolate had neither *aap* nor *ica* genes (Table 2). Among isolates with polysaccharide-enriched biofilms ($n = 14$), the *ica* gene was present in all of them, including 12 (85.7%) isolates that also carried the *aap* gene. As mentioned before, we were not able to determine the composition of biofilms formed by 17 out of the 50 isolates. These isolates displayed all combinations of *ica* and *aap* possible, but the majority (41.2%) of them had only the *aap* gene, followed by isolates that presented only *ica* (23.5%) and then isolates

Table 2
Biofilm matrix composition among different genotypes of *ica* and *aap* in *Staphylococcus epidermidis* isolates.

Biofilm matrix composition	*n* (%)				
	ica+ *aap*−	*ica*+ *aap*+	*ica*− *aap*+	*ica*− *aap*−	Total
Proteic total	0	3 (30)	6 (60)	1 (10)	10 (20)
Proteic in TSB	0	3	1	0	4
Proteic in 1% glucose	0	0	5	1	6
Polysaccharidic total	2 (14.3)	12 (85.7)	0	0	14 (28)
Polysaccharidic in TSB	0	7	0	0	7
Polysaccharidic in 4% NaCl	2	5	0	0	7
ND	4 (23.5)	2 (11.8)	7 (41.2)	4 (23.5)	17 (34)
NP	1 (11.1)	0	2 (22.2)	6 (66.7)	9 (18)
Total	7 (14)	17 (34)	15 (30)	11 (22)	50 (100)

ND = not defined; NP = nonproducer.

that had neither of the two genes (23.5%). Out of all 50 isolates analyzed, 9 did not produce biofilms in any of the conditions tested. Most of these nonproducers (66.7%) did not carry *ica* or *aap* genes.

3.4. Gene expression under osmotic stress

To understand the expression of genes associated with biofilm formation under osmotic stress, we extracted RNA from biofilm cells of a *ica*$^+$ *aap*$^+$ *S. epidermidis* isolate (810) and performed qPCR for *ica* and *aap* genes under different conditions. Isolate 810 was selected because it only formed biofilms under osmotic stress and because it produced a strong polysaccharide biofilm under both osmotic stress conditions (NaCl or glucose). Our data revealed that both genes were upregulated during growth in culture medium containing additional NaCl when compared to growth in glucose-containing culture medium. The magnitude of this induction was around 3-fold for *aap* and 14-fold for *ica* (Fig. 1C). Furthermore, after growth in medium with additional glucose, *aap* was more highly expressed than *ica*, and the opposite trend was observed after growth with additional NaCl, with higher *ica* expression.

4. Discussion

S. epidermidis is the most abundant species in the skin and nasal microbiome. Its ability to be a successful opportunistic pathogen is mostly due to the rupture of the skin integrity using invasive medical devices and increasing selective pressure by antimicrobial usage (Otto, 2009). During their lifecycle, it is likely that *Staphylococcus* cells will encounter osmotic stress. Members of this genus have a remarkable ability to grow at high-osmolarity conditions, and plating these microorganisms at 7.5% NaCl has been a routine method to select for *Staphylococcus.* This osmotolerance probably supports *Staphylococcus* growth and survival in human skin and mucous membranes (Price-Whelan et al., 2013). High glucose concentrations can also be found in the body, and *Staphylococcus* spp. could be implicated in associated infections. In fact, patients with hyperglycemia (up to 0.3% glucose) are known to have an increased risk for implant-associated bacterial infections (Waldrop et al., 2014).

Neonates admitted to NICUs are particularly more susceptible to *S. epidermidis* infection, which can quickly evolve to life-threatening conditions such as bloodstream infections (Brito et al., 2014; Wójkowska-Mach et al., 2014). In this work, 50 *S. epidermidis* isolates from neonates admitted to NICUs in Rio de Janeiro and previously studied by our group (Salgueiro et al., 2017) were further characterized to determine the impact of osmotic stress on biofilm production, expression levels of *ica* and *aap* genes, and composition of biofilm matrices. The results suggest that the type of biofilm formed is associated with bacterial growth conditions, important aspect to help us understand the dynamics of this important virulence factor.

According to Van Kerckhoven et al. (2016), the *ica* operon is present in most *S. epidermidis* clinical isolates. However, biofilm formation can take place regardless of the expression of these genes, mostly due to the presence of Aap and other proteins that can substitute PIA during biofilm formation (Speziale et al., 2014; Van Kerckhoven et al., 2016). Moreover, the conditions and mechanisms behind the selection of the gene expressed during biofilm formation are not fully understood. In our study, among the 17 isolates that had both genes, 7 (41.2%) produced moderate or strong biofilms in all conditions, and osmotic stress enhanced biofilm formation for other 9 (52.9%) isolates. Only 1 isolate did not produce biofilm in any condition, indicating that the presence of both genes correlates with a stronger biofilm production. Furthermore, we observed that even in these efficient biofilm-producing isolates, osmotic stress could induce stronger biofilm formation. Regarding the isolates that only presented the *ica* gene ($n = 7$), osmotic stress was able to further induce biofilm production in most of them (85.7%), and addition of glucose had the biggest impact. Among the isolates that were positive for *aap* only ($n = 15$), again, the majority of them (86.7%) responded to at least one of the osmotic stress conditions by increasing biofilm formation. Therefore, osmotic stress greatly enhanced biofilm formation by *S. epidermidis* isolates, and in general, glucose had the biggest effect, independently of the genetic context. Van Kerckhoven et al. (2016) showed that all *S. epidermidis* isolates that produced a strong biofilm were *ica*A$^+$, whereas the majority of *ica*A$^-$ isolates exhibited weak biofilms. However, not all *ica*A$^+$ isolates produced a strong biofilm, suggesting that *ica*A is an important gene for biofilm formation but also that its presence is not always sufficient for the formation of a strong biofilm. Los et al. (2010) also described that the *ica*$^+$*aap*$^+$ genotype is correlated with strong biofilm production, indicating an additive effect of both genes on the intensity of the biofilm produced. In our study, among the 50 *S. epidermidis* neonatal isolates tested, 11 (22%) had neither *ica* nor *aap*; of these, 4 isolates (36.4%) were able to produce biofilms after osmotic stress induction. The ability of *ica*$^-$*aap*$^-$ isolates to produce biofilms after concomitant addition of NaCl and glucose in growth media was also described by another group while studying *S. epidermidis* colonization isolates (Los et al., 2010). Biofilm production by isolates without *ica* or *aap* could be explained by the presence of other genes, such as *bhp* or *embp*, which also encode proteins involved in biofilm formation (Harris et al., 2016).

A few previous studies have described the impact of growth on media supplemented with either NaCl or glucose on biofilm formation in limited numbers of *S. epidermidis* isolates (Barbieri et al., 2015; Linnes et al., 2013). Barbieri and coworkers demonstrated that biofilm production for most (85.7%) *S. epidermidis* isolates was significantly induced by the presence of glucose (1%), whereas supplementation with 4% NaCl enhanced biofilm production in only 14.3% of the isolates (Barbieri et al., 2015). In another study, it was observed that concomitant supplementation with glucose and NaCl could maximize transcription of the *ica* operon in *S. epidermidis* (Conlon et al., 2002).

Although some studies reported the ability of *S. epidermidis* clinical isolates to produce more biofilm than commensal isolates (Harris et al., 2016; Juárez-Verdayes et al., 2013), little is known about the differences between these 2 types of strains. In our study, we observed a higher prevalence of biofilm producers among isolates from nasal colonization compared to isolates from bloodstream infections. However, osmotic stress impacted biofilm formation by both groups of isolates in a similar manner. Further studies should be performed to better understand this potential difference in the ability of *S. epidermidis* isolates from different clinical sources to produce biofilm.

The chemical composition of biofilms was evaluated for isolates with increased biofilm production in the presence of glucose or NaCl, with either a moderate or strong phenotype ($n = 14$). Even though previous studies suggest that the PIA polysaccharide is the main component of *Staphylococcus* spp. biofilms (Otto, 2013), out of the 14 selected *S. epidermidis* neonatal isolates, only 2 (14.3%) were characterized as having polysaccharide-based biofilms. On the other hand, 7 (50%) presented a mostly protein-enriched biofilm, indicating that, with the addition of glucose to the media, isolates tend to produce protein-enriched rather than polysaccharide-enriched biofilms. In a recent study, Barbieri et al. (2015) observed that during growth on media supplemented with 1% glucose, 6 of the 7 *S. epidermidis* isolates from breast peri-implant infections produced protein-enriched biofilms, whereas the addition of 4% NaCl induced the production of a polysaccharide-enriched biofilm by only 1 of the isolates. The nature of biofilm composition for the remaining 5 (35.7%) isolates from our study could not be determined since their biofilms were similarly affected by $NaIO_4$ and proteinase K treatments. The undetermined nature of these biofilms can be due to several factors, such as the presence of eDNA as an important component of the matrix or a mixed chemical nature of the extracellular matrix produced (Qin et al., 2007). Further studies assessing the impact of DNase on biofilms formed by these *S. epidermidis* isolates should be performed to determine if eDNA is a major component of their biofilm matrices.

The expression of *ica* and *aap* was evaluated on biofilms formed by 1 *S. epidermidis* clinical isolate under osmotic stress. This isolate showed a strong polysaccharide-enriched biofilm in both osmotic stress conditions (NaCl and glucose) but not in TSB medium alone. Since the *ica* operon encodes for PIA, a major component of polysaccharide-enriched biofilms and that is mostly induced by the addition of NaCl (Barbieri et al., 2015), it is reasonable to hypothesize that the expression of this genetic locus may be induced by NaCl. In our study, both *ica* and *aap* were expressed at higher levels during growth in NaCl. Addition of NaCl at 4% has been shown to increase expression of *ica* and *aap* in half of the *S. epidermidis* isolates from cerebrospinal fluid (2 out of 4) (Stevens et al., 2009). According to Stevens et al. (2009), besides PIA production, protein factors may strongly contribute to the development of mature biofilms, although Aap is not always a contributing factor to biofilm formation when there is a completely functional *ica* operon present.

S. epidermidis is greatly associated with bloodstream infections, mainly in high-risk populations such as premature neonates, and its ability to produce biofilms facilitates the colonization of diverse surfaces, increasing its persistence. This study showed that osmotic stress conditions improved biofilm production in a collection of *S. epidermidis* isolates from neonates from both colonization and infection sites. Moreover, most of the biofilms could only be detected after growth under these conditions, suggesting that the growth medium correlates with the type of biofilm produced. Therefore, *S. epidermidis* biofilm expression can be influenced by environmental factors, and the characterization of this phenomenon is critical to help us understand the dynamics of this important virulence factor in *S epidermidis* causing clinically-significant infections in a high-risk population.

Declarations of interest

Declarations of interest: none.
Ethical approval: not required.

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